
Chapter 8

Bud Extracts as New Phytochemical Source for Herbal Preparations — Quality Control and Standardization by Analytical Fingerprint

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Additional information is available at the end of the chapter

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1. Introduction

1.1. General, economic, and social notes

1.1.1. Historical background of the gemmotherapy method

The use of buds for therapeutic purposes dates back many centuries. Ancient Indian medicine (Ayurveda) used, and still uses, plant buds for therapeutic purposes. In Western Europe, Galen (second century A.D.) prepared Acopton, one of the most renowned vulnerary balsams of that time, by soaking poplar buds in olive oil for three months. Nicolas De Myrepse, a medieval Greek physician, revived this idea and formulated the famous “Unguentum Populeum.”

Buds and sprouts were identified with the persistence of the life cycle, which renewed itself each spring [1, 2]. Paracelsus realized that the different parts of a single plant had distinct properties [3].

In ancient times, physicians, botanists, and naturalists were interested in defining plants and their derivative properties. According to Theophrastus, herb quality was dependent on a series of factors, including plant age, collection method, used plant part, and geographical origin, as well as preparation methods and storage conditions of the final extracts. In the first century AD, Plinio il Vecchio warned his readers about the herbal preparation quality in relation to possible substitutes, pollutants, deterioration due to age, pests, or inadequate storage. Two centuries later, Galen emphasized the importance of distinguishing good quality products on the basis of sensory tests, drug potency, and geographical origin. During the Renaissance,

scientists and scholars also performed medicinal plant quality control through studies and experiments on fresh plants. Safety, quality, and efficacy, therefore, have always been the key factors distinguishing a therapeutic plant-based product, and over time they have become increasingly complex and important criteria.

Pol Henry was the first researcher who systematically studied bud-preparations. He is therefore considered to be the true “founding father” of gemmotherapy in its current form. He was the first to have the idea of using extracts derived from meristematic tissues rather than adult plant parts for human therapy and proposed a therapeutic method based on analogical protocols. He conducted a series of clinical trials on humans and animals to establish the psychopharmacological effects of, initially, some twenty or so gemmotherapy remedies. The results of his initial works were published in the “Archives Homeopathiques de Normandie” in 1959. Since then, many scientific publications have confirmed the validity of this therapeutic method [8, 9].

Gemmotherapy is a therapeutic method belonging to the field of Biotherapy, which, based on the analogical–biochemical principles of biological drainage, uses hydroglyceroalcoholic solutions of macerated fresh plant extracts in the first decimal dilution for therapeutic purposes [1, 6]. These extracts consist of meristematic tissues, such as buds, sprouts, young stems, rootlets, catkins, the inner root cortex, the young branch cortex, sap, seeds, and other meristematic plant tissues in the growth phase [10]. To express the concept at a taxonomic level, it would be more correct to describe the method as “meristemotherapy,” since the used plant tissues are of meristematic origin.

Gemmotherapy/meristemotherapy is therefore a medical method that uses extracts of fresh plant tissues in the growth phase. Sprouts, buds, and other meristematic plant tissues retain all the anabolic faculties of the primitive plant cell, which is capable of developing all the potential no longer found in the adult plant. This therapeutic plant medicine belongs to “renewed phytotherapy,” a branch of the biotherapeutic medicines classed between allopathic medicine and homeopathy. It was relatively unknown until a few years ago, but it is now growing considerably, in Italy and elsewhere [13]. The reason for this popularity lies in the fact that bud-preparations are easy to administer: they are sold ready-for-use and simply need to be diluted in water.

1.1.2. New interest and current diffusion of phytotherapy

Phytotherapy has thus developed from knowledge that was handed down over the course of millennia and has been confirmed, modified, or disproved through many pharmacological studies and clinical trials (Figure 1). It has therefore become a fully fledged medical discipline, since the knowledge gleaned from folk medicine has since been subjected to methodical scientific assessment in order to provide evidence of its efficacy [1, 15].

Progressively deteriorating environmental quality, pollution, excessive reliance on technology, continuous innovation of products and synthetic drugs perceived as alien to the organism, the indiscriminate use of many chemicals, and the change in the concept of health, which is no longer seen merely as the absence of disease but as a general sense of well-being, have

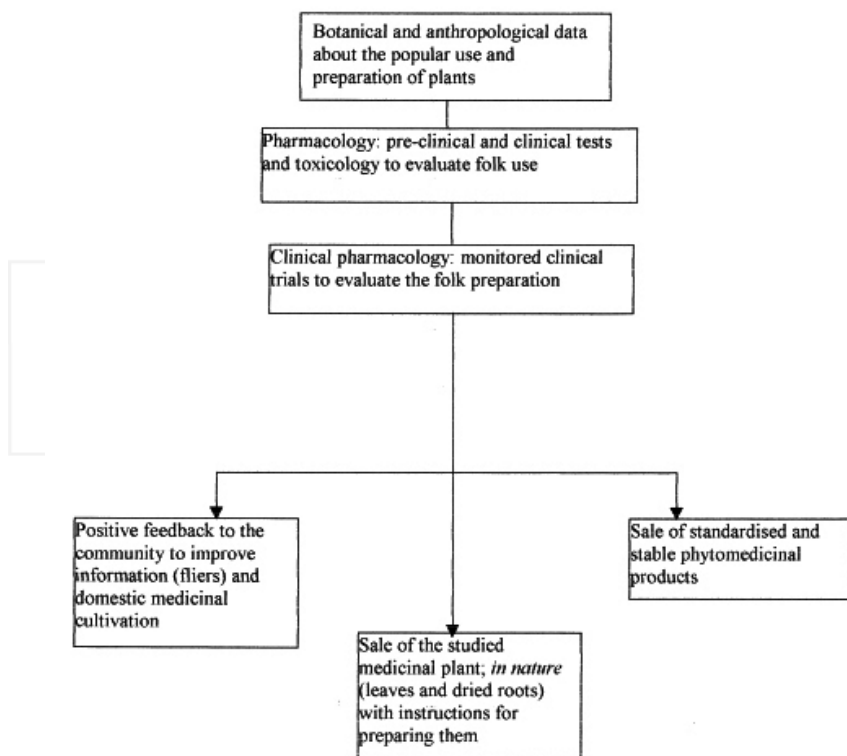


Figure 1. Methods for pharmacological validation of the popular use of medicinal plants [12].

driven “modern people” to “return to nature.” The use of phytotherapy has considerably increased in recent years, thanks to important studies confirming the medicinal properties of plant extracts and the continuous growth in scientific knowledge of their positive effects on humans and animals.

However, the use of plants for therapeutic purposes should be assessed by both the treating physician as well as the patient; the bioactive substances found in plants and their derivatives can also cause unwanted side effects, allergic reactions, or interactions with other plants or medicines [17]. For some diseases, moreover, no “miracle plants” are currently available, and so official medicine is the only option. They can, however, replace or complement synthetic drugs for several functional and somatic disorders. For example, many laxatives, painkillers, and tranquilizers can be replaced by a milder treatment based on medicinal plants [18].

The recent ISTAT multipurpose survey “Health conditions and the use of health services,” conducted in 2005, has a section on the use of nonconventional therapies, which are also referred to as alternative, complementary, or traditional (at times ambiguously). The survey was carried out on a sample of approximately 60,000 households and showed that 13.9% of

the Italian population (7.9 million) used nonconventional medicine during the three years preceding the survey, particularly homeopathy (7%), manual therapies (6.4%), and phytotherapy (3.7%). There was also a clear prevalence in the north of the country (North-east Italy 21.9%, North-west Italy 17.9%, Central Italy 13.6%, Southern Italy 5.4% and the Italian islands 7%). The users were mostly women (15.8% compared to 11.2% of men) between thirty-five and forty-four years of age.

The inclination to use nonconventional treatment methods increases with the level of education: 18.7% of people with a degree or diploma, 13.5% of people with a middle-school diploma, and 9.2% of those with primary school certificates use these methods. Executives, entrepreneurs, professionals, and office workers, in particular, most often used alternative therapies during the period covered by the survey [19]. According to the ISTAT survey, 71.3% of the patients stated that they chose nonconventional medicine because they considered it less toxic than conventional medical treatments.

General public opinion regarding UMs remained divided. However, 48.8% considered them to be effective and 51.2% regarded them as useless. Men were found to be more critical than women in regard to the usefulness of these methods. Most (73.5%) of the people interviewed in 2005 combined both homeopathy and phytotherapy with standard medicines. Specifically, 44.2% of them tried homeopathy and phytotherapy but mainly relied on conventional medical therapies, whereas 29.3% stated that they had combined conventional medical treatments with alternative therapies, relying mainly on the latter; 17% of the questioned people used homeopathy and phytotherapy exclusively, without combining them with other conventional medical treatments.

More recently, Eurispes indicated that 14.5% of Italians used nonconventional medicines in 2011, a decrease of 4% from 2010. Homeopathy remains the preferred therapy (70.6%), followed by phytotherapy (39.2%), osteopathy (21.5%), acupuncture (21%), and chiropractic (17.2%). There is a greater tendency toward regular use of alternative therapies in other European countries: 49% and 46% of the population in France and Germany, respectively, used it regularly, along with 35%, 31%, and 25% of the population in the United Kingdom, Belgium, and the countries of Northern Europe, respectively [20].

There are many reasons for the increasing interest in and availability of nonconventional medicines. Many patients try them because they have not benefited from traditional medical remedies or because they caused side effects. Moreover, the basic UM doctrines are holistic in nature, i.e., they consider the patient in his or her psychophysical entirety. A particular doctor-patient relationship is thereby established, based on the premise that a specific disorder may originate from a multiplicity of factors [3, 21]. The approach to the problem is therefore not confined to the treatment of a particular symptom, but extends to an analysis of the patient's personal and family background.

The growing popularity of these alternative medicine practices is also reflected in the efforts over the last twenty years by the World Health Organization (WHO), and, more recently, by the National Center for Complementary and Alternative Medicine (NCCAM) of the National

Institute of Health (USA) to establish guidelines for methods to research and evaluate the effectiveness of nonconventional medicine [1, 22].

1.2. Bud-preparations

1.2.1. Buds and others meristematic tissues

Research has shown that, in addition to cytoplasmic substances, meristematic tissue cells also contain plant hormones (auxins, gibberellins, cytokinins, abscisic acid, and ethylene), enzymes, aminoacids, trace elements, vitamins, flavonoids [23, 24], and nucleic acids (DNA and RNA), many bioactive compounds are often found only in trace amounts in adult plants [9, 25]. The effectiveness of bud-preparations is due to the combination of all these substances (called “botanicals”), which constitute the bud-phytocomplex.

Plants preserve meristematic cells in their meristematic tissue. The meristem biochemical profile is not comparable to the adult plant organ profile, because plant growth imposes a specialization aimed at specific biological roles [11, 26]. Meristematic tissues are composed of groupings of meristematic cells: the specialized adult tissue cells are subsequently differentiated from these cells. Buds are particular organs produced by the plant to protect these tissues during the cold season: new plant parts will emerge at the end of this unfavorable period from these tissues [25].

The following parts are used in gemmotherapy: buds, sprouts, rootlets, root cortex, young branch cortex, and seeds. Buds and sprouts are the most common tissues used for the bud-preparation production [8, 27].

The buds spend the winter in a sleeping state, which begins with a period marked by decreasing temperatures or a progressive reduction of the photoperiod. The end of dormancy is determined by the exposure of the buds to relatively low temperatures for a certain number of hours (chilling requirement) and by a decrease in the level of abscisic acid, with an increase in cytokinin and gibberellin content. Indeed, in preparation for winter, abscisic acid is produced in terminal buds: this slows plant growth and directs leaf primordia to develop scales to protect the dormant buds during the cold season. It also inhibits the division of cells in the vascular cambium, adjusting to cold conditions in the winter by suspending primary and secondary growth. Gibberellins and cytokinins are also involved in the natural process of breaking dormancy and cell differentiation [25].

The breaking of dormancy is also associated with an increase in organic acid content and respiration in buds. Organic acid content was inversely related to carbohydrate content in developing buds [28].

Moreover, in the early stages of development, the plant tissues contain specific bioactive compounds: later, these molecules are subjected to metabolic transformations, as the removal/adding of hydroxyl groups, diversifying, for example, the polyphenolic patrimony; in this case, recent histochemical tests revealed the presence of three types of polyphenolic compounds in buds, differing in what their intracellular localization is concerned: granular polyphenols, vacuolar polyphenols, and droplike polyphenols. Finally, the sugar moieties are diversified in

mature organs: in buds, the preferred sugar for glycosylation is galactose, while, later, the glycosylation preferentially occurs with rhamnose [27].

1.2.2. Preparation protocols

The official procedure for bud-preparation production is detailed in the monograph “Homeopathic preparations” published in the 8th edition of the *French Pharmacopoeia* and subsequent editions [29].

This book describes the different stages of the extraction and preparation process:

Collection. Meristematic tissues should be collected in their balsamic period, which generally coincides with late winter–early spring, as the greatest concentration of bioactive compounds occurs at this time.

Cleaning. The newly harvested fresh parts are cleaned thoroughly to remove any foreign parts or soil residues.

Measurement of humidity level and dry weight. Samples are placed in a stove at 105°C to dehydrate for few days in order to reach a changeless weight, defined as the dehydrated weight. This operation allows the quantity of ethanol and glycerol to be calculated for the subsequent maceration step.

Grinding. The clean portion of fresh plant material is manually fragmented, using a mortar, for example, in the case of large buds. This operation facilitates extraction by the solvent.

Maceration. The fresh plant material is left to macerate for 21 days in a mixture of 95% ethanol and glycerol in a 1:1 ratio: the solvent quantities are calculated in order to obtain a 1:20 glycerine macerate, so that the final product is 20 times the weight of the raw material in the dry state. The maceration must be performed immediately after harvesting, to prevent the fresh tissue undergoing enzymatic and oxidative degradation.

Decanting, filtration, and pressing. Once the maceration is finished, the mixture is decanted and then filtered. The filter is pressed using a manual press. The product obtained from the pressing is added to the filtrate, which is then left to settle for another 48 hours. Finally, it is filtered once more, obtaining the mother preparation.

Dilution. The macerate is diluted to the first Hahnemann decimal (DH 1): one part of the mother preparation is diluted with nine parts of a mixture containing 50 parts (by weight) of glycerol, 30 parts of ethanol, and 20 parts of water. The mixture is then stirred for optimal mixing of the preparation. The alcohol content normally attained is 38°.

Checks. The final product is checked to assess and determine the smell and taste (by sensory analysis), color (by spectrophotometry), and alcohol content (e.g., by gas chromatography). A further test is useful to detect the presence of any contaminants, such as molds, yeasts, methanol, or 2-propanol.

1.2.3. Relationship between drugs/bud-preparations and phytochemicals

The principle of pure and pharmacologically active compounds was and remains the goal of pharmaceutical-chemical research in this century. Plants have been considered a very important source of active molecules for direct extraction or as a basis for the synthesis of molecules with specific pharmacological properties. Chemists have identified some of these many compounds and extracted them from plants, and physicians have studied their action mechanisms and therapeutic effects. This has allowed the identification not only of bioactive compound classes, such as flavonoids, saponins, terpenes, but also of some single molecules (caffeic acid, ferulic acid, chlorogenic acid, quercetin, and many more). Pharmaceutical chemistry has thus begun to reproduce their fundamental structures, sometimes modifying them to obtain products with therapeutic effects of equal, if not greater, effect.

Conventional drugs contain a single active compound (represented by molecules by synthesis or by plant extraction), which has the purpose of interacting with a specific tissue-target in order to produce a fast and effective result. They are therefore suitable for acute diseases or in deteriorating chronic conditions [21, 33]. Phytotherapy treatments, like synthetic drugs, act on the organism by chemical substances, which have a pharmacological effect [6]. Due to the large quantity of bioactive compounds found in phytotherapeutic products, many of which act synergistically, there is a preference to attribute the pharmacological effect to the “phytocomplex,” rather than to any single active compound, as in the case of standard medicine.

The phytocomplex consists of a combination of different substances, both active principles and other plant components, which contribute to the overall therapeutic effect. The combination of individual components contained in the phytocomplex is able to release a synergistic action as a whole [21] and to reduce the risk of addiction and toxicity, producing a more complete and less drastic pharmacological effect [9] than that of one or a few of its components taken separately.

The main compounds with phytotherapeutic action are the plant secondary metabolites, i.e., compounds without a direct function in regard to growth and development. Unlike the primary metabolites, such as chlorophyll, aminoacids, nucleotides, or simple carbohydrates, they do not have roles in the processes of assimilation, respiration, transport, and differentiation. Although they are widely distributed throughout the plant kingdom, secondary metabolites are normally found in a specific plant species or in a group of related species and are only localized in specific tissues and organs or in particular stages of development [6, 34].

1.3. Main critical points

1.3.1. Final product and supply chain

The current literature on bud-preparations shows a discrepancy regarding the preparation protocols. The *French Pharmacopoeia* requires the bioactive compounds to be extracted in a mixture consisting of ethanol and glycerol in equal parts. It also requires the “mother solution” to be diluted with a mixture of water, alcohol, and glycerol in a 1:10 ratio (one part of base macerated in nine parts of this mixture).

According to some experts, however, if the base preparation is diluted in a ratio of 1.5:10 (a more concentrated product), the bud-preparation will provide a higher number of clinical benefits [8]. Other herbal companies propose yet another maceration method, divided into two steps. During the first step, which lasts for a week, the plant material is macerated in ethanol alone. In the second step, after seven days, a solution of water (revitalized through a particular process) and glycerol in equal parts is added. In other cases, the bud-preparation is prepared by macerating the plant material (100 g) in a 1 L mixture with different extraction solvents, in a 1:2 ratio, while stirring vigorously. After a month, the macerate is filtered and the solvent removed with a rotary evaporator [24].

Another critical point concerns the botanical nomenclature showed in the bud-preparation package. It is not uncommon to find inaccuracies regarding the species names, as in lemon and chestnut herbal products. The latter, for example, is named as *Castanea vesca*, and this scientific name was replaced many years ago by *Castanea sativa*. In the European market, most of the plant material (80%) for herbal preparations is harvested in the wild, but also when harvested from specialized cultivations herbal products are only generally labeled with the species name without any regard to genotype (Figure 2).

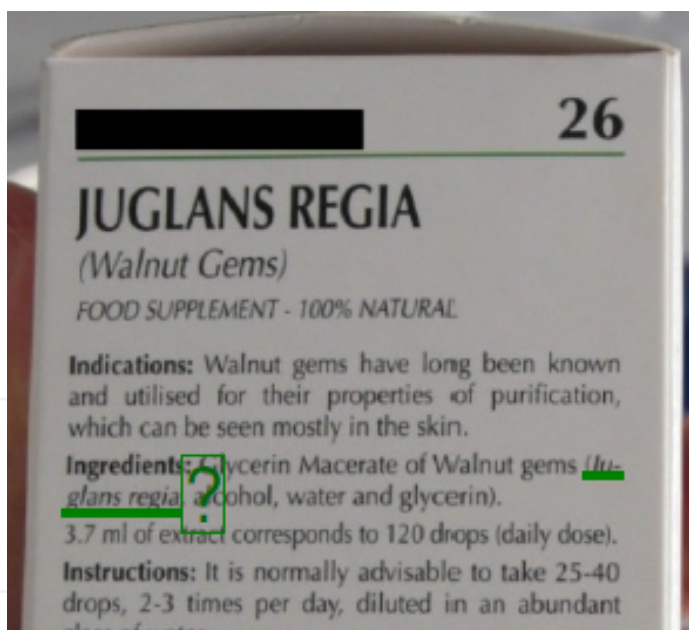


Figure 2. No cultivar information is reported on the bud-preparation packages.

No plants are specifically grown for the phytotherapeutic market, and there is a lack of basic knowledge, i.e., researches on the potential qualitative and quantitative differences between different genotypes of a species.

Finally, the bud-preparation regulatory classifications are ambiguous (Figure 3): they have been associated with homeopathic products by the *French Pharmacopoeia*, based on their dilution method, but they can also now be associated with the food supplement category.



Figure 3. Bud-preparation regulatory classification is still contradictory and ambiguous.

1.3.2. Analytical techniques

Compared to other phytotherapeutic products, bud-preparations are currently affected by critical aspects, due to the total lack of analytical studies and comparative references to other research experiences at national and international levels [38].

An important issue concerns the analysis methods. Technological innovation in the analytical instruments has allowed increasingly sophisticated qualitative and quantitative assessments of the plant extract chemical composition. Chromatographic techniques are mainly used to analyze herbal preparations with some analytical problems related to the complexity of the initial matrix and the nature of the extraction solvents, because glycerol does not allow for the application of certain commonly used analytical techniques. Thin Layer Chromatography (TLC), for example, which is normally used to analyze mother tinctures, cannot be applied to

bud-preparations, because the glycerol does not allow the analyte migration to the chromatographic plate. With paper chromatography and TLC, very small samples could be analyzed, and the resolution and reproducibility could be improved. However, quantitation is still inadequate and the resolution of similar compounds is difficult.

Chromatography includes a range of different instrumental techniques, from the simplest to the most sophisticated (Table 1).

Chromatographic Method	Separation Mechanism
Liquid–solid chromatography	Adsorption
Paper	(Adsorption), partition
Gas liquid chromatography (GLC)	Adsorption, partition
Thin-layer chromatography (TLC)	Adsorption, partition
High-performance liquid chromatography (HPLC)	Adsorption, partition
Ultra-performance liquid chromatography (UPLC)	Adsorption, partition
Supercritical fluid chromatography (SFC)	Adsorption, partition
Liquid–liquid chromatography (LLC)	Partition
Countercurrent chromatography (CCC)	Partition
Ion-exchange chromatography (IEC)	Ion exchange
Capillary electrophoresis	Charge
Ion-pair chromatography	Ion pair formation, ion interaction
Hydrophobic interaction chromatography (HIC)	(Adsorption), partition
Size exclusion chromatography (SEC)	Size of analyte
Affinity chromatography	Biological affinity

Table 1. The major chromatographic methods used in phytochemistry [40].

Their common feature is a separation achieved through the flowing of a mobile phase, the eluent, containing the analytes to be separated, over a fixed phase, the adsorbent, which exerts a selective action on the compounds. Chromatography offers very powerful separation ability, such that the complex chemical components in herbal extracts can be separated into many relatively simple subfractions. Furthermore, the recent approaches of applying hyphenated chromatography and spectrometry such as high-performance liquid chromatography–diode array detection (HPLC–DAD), gas chromatography–mass spectroscopy (GC–MS), capillary electrophoresis–diode array detection (CE–DAD), HPLC–MS, and HPLC–NMR, can provide additional spectral information. This is very helpful for the qualitative analysis and for the on-line structural elucidation.

More specifically, High-Performance Liquid Chromatography is currently the most widely used analytical method, combined with different detectors (diode arrays, mass spectrometry,

and fluorescence). All the compounds in herbal preparations can be analyzed by HPLC because this popular analytical method is easy and not limited by compound volatility and stability.

It is a chemical–physical separation method: different molecules distribute themselves, according to determined relationships, between two separate and distinct phases, one fixed into a column and the other mobile. In this case, there is a real molecules separation, while in other analytical methods, as spectrophotometry, the single compounds remain united and the separation consists in highlighting the different analyte interaction with the radiation. In the analysis of herbal products, reversed-phase (RP) HPLC columns are the most used stationary phases for compound separation. Different factors are involved in chromatographic separation as mobile phase chemical composition, pH of solvents, pump pressure, flow rate, and selected wavelengths [44].

Finally, the implementation of fast and reliable methodologies for analysis based on GC–MS, HPLC–UV–MS, etc., made possible the identification and quantification of known compounds in plants and other organisms [16].

2. Phytochemical composition and quality of gemmotherapy products

Gemmotherapy can provide effective therapy only through the availability of suitable plant extracts that are as uniform as possible. A great deal of information is necessary to ensure the herbal preparation’s safety and quality (Table 2).

Botanical source	1. Identity: family, genus, and species of source plant (with authority) and, if relevant, variety and chemotype; common names
	2. Part(s) of plant used
	3. Geographic origin
Growing conditions	1. Wild or cultivated
	2. Use of good agricultural practice
	3. Site and time of harvest stage of growth at harvest
	4. Postharvest treatment (drying, fermentation, etc.)
	5. Storage conditions
	6. Phytosanitary measures pre- and postharvest (including use of and limits for pesticides)
Raw material (e.g., dried plant material)	1. Specifications according to standard reference (e.g., herbal pharmacopoeias)
	2. Identity tests (macroscopic, microscopic, FT-IR, TLC, GC, HPLC, etc.)
	3. Quantitative tests (especially constituents relevant for efficacy and/or toxicity—not necessarily the same)

Process applied to starting material	1. Steps in preparation (e.g., separation, extraction processes, solvents)
	2. Methods used
	3. Specific precautions addressing, e.g., light/temperature sensitivity, oxidation
Botanical preparation	1. Standardization criteria (markers: active constituents, other relevant components; plant extract ratio)
	2. Specifications: levels and range for markers
	3. Physicochemical properties of relevant constituents; stability
	4. Purity criteria by chain control or analysis; microbiological, mycotoxins, pesticides, environmental contaminants
	5. Nature and level of excipients; formulation methodology
	6. Storage conditions
End product (food or supplement containing the botanical preparation)	1. Fate in food or formulated product
	2. Fate on industrial processing and/or domestic preparation for consumption

Table 2. Information relating to herbal product identification, characterization, and standardization [50].

2.1. Genotype and bud phenological stage

The bud-preparation quality begins in the field through the species identification before bud collecting. Species identification should be done by botanists or agronomists, as the diagnostic features typically used in botanical identification (leaves, flowers, etc.) are not usually present in the bud-harvesting season. However, some dichotomous keys allow trees to be identified in winter, through bud color and shape analysis [8]. Identification is an important step in the wild plant collection, even if it is often overlooked by the herbal companies: in the European market only 20% of the plants for herbal products are collected in germplasm repositories. Moreover, an individual plant species may have different cultivars, with different genetic characteristics, chemical compositions, and health effects on final product efficacy and consumer’s health [52].

The buds are picked up in the early spring, when they emerge from winter sleeping. This time is known as the “balsamic period,” a specific phenological stage in which the meristems have high concentrations of bioactive compounds [8]. The balsamic period, specific to each plant part, depends on the plant vegetative cycle; it can also vary because of different sampling sites and years. Tree species development is also influenced by natural factors (endogenous or exogenous), as well as by human factors. These factors can influence the plant organ chemical composition (and the respective herbal products).

2.2. Pedoclimatic conditions and agrotechnique

The herbal preparation quality is not only dependent on intrinsic characteristics (genetic factors), but is also closely linked to the collecting-area pedoclimatic conditions. Indeed, plants

growing in more suitable areas have greater productive potential in terms of product quantity and quality [56]. In the case of plant material for bud-preparation production, knowledge of environmental characteristics, such as the best pedoclimatic conditions for the plant development, can help choose the best raw material to be used for bud-preparations [3, 12].

Each species requires its own best soil and climate, in terms of altitude, latitude, mean temperature, average annual rainfall, light availability, and physico-chemical soil properties. Climatic conditions show a direct effect on plant physiological processes and phenology, such as growth, flowering, and fruit ripening, thus they can also affect the availability of essential metabolites for the active compound biosynthesis [56, 57]. A plant can almost completely lose the ability to synthesize specific bioactive molecules outside of its own habitat [25]. The use of advanced agrotechniques together with a dedicated genetic improvement allow tree species to fit better with the cultivation site and can also improve the herbal product final quality [57].

2.3. Extraction methods

However, the herbal preparation quality is also determined by the following processing and storage procedures. The extraction technique is an important processing step that requires specific chemical and analytical knowledge. The extraction method and the used solvents show a high influence on the extract releasing and bioactivity. Each plant organ, due to the different chemical composition, has its own specific extraction method. The extraction method and its effectiveness depend on the solvent nature, which is linked to the different chemical compound polarities [24, 35], and to the extraction time and temperature [58].

Due to bud-preparation variability and complexity, it is very difficult to control their product quality: the key factors in achieving this objective are determination of the chemical composition and standardization. The definition of an analytic fingerprint allows for qualitative and quantitative phytocomplex component evaluation: for this reason, botanical fingerprints become an effective tool for quality control [44]. This quantity should not be below a minimum level; otherwise the product will not have the proper therapeutic effect.

3. Analysis of phytochemicals in herbal products

3.1. Analytical methods

Identification and quality control of the herbal material can be performed through macroscopic and microscopic techniques and the use of marker compounds [41]: a marker compound is a chemical constituent of a botanical raw material, drug substance, or drug product that is used for identification and/or quality control purposes, especially when the active constituents are not known or identified. The active compounds are responsible for the intended pharmacological activity or synergistic therapeutic effects [35]. It is important to assess the phytochemical composition of herbal medicines in order to study pharmacological and clinical properties (bioactivity and possible side effects) of these products and their bioactive constituents. [44].

Sample preparation is the most important step in the development of analytical methods for the analysis of botanicals and herbal preparations. The basic operation included steps, as prewashing, drying of plant materials or freeze drying, grinding to obtain a homogeneous sample and often improving the kinetics of analyte extraction: the official extraction method, detailed in the *French Pharmacopoeia*, should be chosen in order to produce commercial preparations.

After bioactive compound extraction, in order to evaluate the entire pattern, chromatography combined with a suitable detection technique offers a powerful tool for separating the individual compounds and developing a characteristic profile of the sample, called a fingerprint. In particular, High-Performance Liquid Chromatography (HPLC) is the most popular analytical technique for analyzing herbal products: HPLC is an easy to operate, fully automatable technique with high resolution, selectivity, and sensitivity. One of the main advantages of HPLC is the possibility to couple the technique to different detectors: combining a chromatographic separation system on-line with a spectroscopic detector has become the most important approach for the identification and/or confirmation of the identity of target and unknown chemical compounds in herbal preparations. For most (trace-level) analytical problems in the research field of herbal medicines, the combination of column liquid chromatography with a UV-vis or a mass spectrometer (HPLC-DAD, LC-MS, respectively) becomes the preferred approach for the analysis of herbal medicines. HPLC coupled with UV photodiode array detection (DAD) allow the running of a chromatographic separation with simultaneous detection at different wavelengths. The UV spectra of bud-products give useful information on the type of constituents: indeed, new instruments allow the recording of UV spectra of compounds, which can be performed automatically when screening for known constituents [40]. The data obtained from hyphenated instruments are the so-called two-way data (one way for chromatogram and the other way for spectrum), which provide much more information than the classic one-way chromatography in order to obtain a complete chemical fingerprint. If hyphenated chromatography is further combined with chemometric approaches, clear pictures might be developed for chromatographic fingerprints obtained.

3.2. Statistical data analysis

After selecting an appropriate technique to analyze the bud-preparation samples, an adequate method should be developed, optimized, and validated with a multivariate statistical approach, as the Design of Experiments (DoE) or experimental design. In the multivariate approaches, a predefined number of experiments are performed according to a well-designed experimental setup in order to examine several factors simultaneously. The factors having the most effect on the outcome of a chromatographic analytical method are determined from screening designs: these factors included, amongst others, mobile phase composition, buffer concentration and pH, detection wavelength, column temperature, injection volume, sample concentration, and column type.

Due to the complexity of herbal medicines, and in particular bud-preparations, it is necessary to consider many factors for their fingerprint evaluation: some minor differences between strongly related genotypes may not be observed, but can largely affect the health of the patient.

Classification of, and discrimination between herbal species is an important strategy in the quality control of herbal products [71]: the quality of bud-preparations is closely related to the chemical constituents and their concentration, and might vary slightly according to differences in climate, cultivation conditions, harvest time, drying, and storage [42]. To analyze the large number of generated data, a variety of analytical methods prove to be useful and versatile tools for the extraction, visualization, and interpretation of the information. Pattern recognition methods as Principal Component Analysis (PCA) allow better visualizing of the information that is included in the fingerprints. Exploratory data analysis is easier if represented as a multivariate data table as a low-dimensional plane. The original variables are transformed into latent variables summarizing the systemic patterns of variation between the samples. For this reason, PCA is often applied to chemical data expressed by bud-preparation fingerprinting.

4. Product quality

4.1. Safety

Although botanicals have played a role in the marketing of health products to reduce age effects, there is an increased interest today due to their perceived health benefits. Not only do consumers increasingly take charge of their health, but the scientific information and understanding of the beneficial health effects of bioactive substances in food, functional foods, and food supplements have improved. Increasing use of these products has also led to concerns about their actual safety.

The safety assessment of botanicals and botanical preparations in food and food supplement is complicated by, amongst others, the variability of composition and it should at least involve:

- The origin and characterization of the raw material and its quality control
- The intended use and consequent dosage
- History of use and exposure
- Product comparison(s)
- Toxicological information gathering
- Risk characterization/safety assessment

An increasing number of botanical products are used as food ingredients or supplements and these are a commercially important part of the health and food market. Botanical products may have different formulations, from whole foods to pharmaceutical-like preparations in unit dose form, as tablets, capsules, or drops [75].

The regulatory position with regard to food supplements is unclear (food or medicine?), and the increasing number of products in the food market raises concern about the safety assurance of these products, as many cases of intoxication with the use of botanical products are reported [50]. In some cases, these have resulted from contamination with other plant

species. In addition, different parts of the plant source may have widely different concentrations of toxic constituents, e.g., pyrrolizidine alkaloids [76], and climatic and agronomic differences may lead to great variability in composition [53]. Since compositional variability of bud-products may occur, it is important that the safety-evaluated botanical material is representative of the commercial material and has adequate specifications of identity and purity. Also, identification of the plant material, details of all the applied processes and compositional standards for the finished product are pivotal. Data should be based on a sufficient number of batches to permit the assessment of the natural variability under different agronomic conditions and establishment of appropriate specifications, and sampling plans should take the variability into account [33].

For some botanical products, good epidemiological and clinical human studies addressing both efficacy and safety may be available. However, if a herbal extract was to be used as a food supplement, the safety evaluation may be focused on the effects of the fractionation process, i.e., nature and concentrations of bioactive compounds relative to the traditional source and likely effects on bioavailability and intake [20].

Hazard identification (adverse effects and adverse effect nature) and hazard characterization (human-relevant dose levels) are necessary to determine the nature and level of risk associated with a particular level of herbal preparation exposure (intake) [75]. The risk assessment/safety evaluation relates to the material as consumed, thus data applicable to the finished products are of major importance. Therefore, detailed analytical fingerprint and other data are required to give assurance that the tested material provides relevant information, bearing in mind the possibility of matrix effects, e.g., on bioavailability, absorption, and pharmacokinetic behavior [60]. There is need of adequate chemical information related to all the phenological stages and to all the potential biological properties of the botanical material, also evaluating the possible botanical interactions with pharmaceuticals, aspects not normally considered for food additives [50].

4.2. Standardization

Scientists have been proceeding in a systematic way in order to validate the claims of many medicinal plant species. For some symptoms and ailments, this may be fairly easy to prove, but in more complex health conditions, the situation becomes a bit complicated. Nonetheless, medicinal plant extracts are showing a great deal of promise, even in instances of complexity of illnesses.

Millions of dollars are spent each year on herbal products that are marketed as food supplements, but in reality very few know, chemically, what they are purchasing or using. Very often the dosage varies from the different brands of the same herbal product. The chemistry and efficacy of many of these plants are relatively unknown and there is a chance of toxicity or overdose until the secondary compounds are known and understood [74, 77]. There is a tendency in the United States and in Europe to regulate and license this market, and this has led to greater and more effective use of these important medicinal plants. There is also general agreement that chemical standardization is the way forward in order for herbal remedies to be prescribed to patients who seek to be treated with medicinal plants [2].

Standardization is a method for assuring a minimum level of bioactive ingredients in the extracts and is becoming increasingly important as a means of ensuring a consistent supply of high-quality phyto-pharmaceutical products. It can be defined as the establishment of reproducible pharmaceutical quality by comparing a product with established reference substances, and by defining minimum amounts of one or several compounds or groups of compounds [35]. In general, in the field of phyto-medicines, standardization is only applied to extracts and not to diluted products. Standards for bioactive molecules to be used in medicinal products may be found in monographs and/or pharmacopeias. A pharmacopeia is a collection of quality standards for medicines and their components. In order to obtain marketing authorization for a medical product, the ingredients or the medicinal product must generally comply with a pharmacopeial standard. Thus, pharmacopeial standard may provide guidance on acceptable purity criteria for that ingredient.

It is accepted that concentration or dosages are very important because herbal medicines (in common with conventional medicines) contain biologically active substances that may produce nontrivial side effects when taken in excessive doses. Very low doses, on the other hand, may have no therapeutic value. Moreover, plant material is often highly variable, so that a minimum concentration or a concentration range is often used rather than an exact level. An upper limit is necessary with highly active or potentially harmful ingredients, as most plants have a wide therapeutic window (e.g., a toxic compound is considerably higher than the therapeutic dose). In the case of compounds with a narrow therapeutic window, chemical entities are favored, as opposed to extracts. Standardization also allows comparison of the clinical effectiveness, pharmacological effects and side effects of a series of products (e.g., against a placebo) [5]. Standardized products provide more security and increase the level of trust people have in herbal drugs.

Different kinds of extracts should be considered depending on standardization:

- **Standardized extracts:** are those for which the bioactive constituents (single or groups) are known. They can thus be standardized to be a defined content of the active constituents giving a clearly defined amount of an active natural product.
- **Quantified extracts:** are those having constituents with known therapeutic or pharmacological activity. Groups of compounds likely to have the desired pharmacological activity are unknown, but are not solely responsible for the clinical efficacy of the extract. The pharmacopeia monograph must define a range of content of the selected constituent(s) some of which are lead compounds.

Standardization by blending different batches of a herbal drug before extraction, or by mixing different lots of herbal drug preparation, is acceptable but adjustment using excipients is not acceptable [22, 77].

4.3. Quality control

Quality control for natural medicine begins in the field and ends with a safe and effective product being delivered to the patient, followed by post-marketing pharmacovigilance: the evaluation of genetic, agronomic, and environmental parameters is the first critical step in all

the quality control supply chain. It is an evolutionary process. The World Health Organization has recognized this, and developed a series of guidelines to assist nations to develop their strategies for the quality control of herbal medicines.

Phytochemistry has an important and pivotal role in the implementation of each of the quality control steps. Each step requires knowing what is in the plant in terms of bioactive compounds, knowing what is happening to those constituents during the processing of the plant, and assuring appropriate analysis methods when materials are being tested biologically, and eventually delivered to the patient in a finished product [33].

There are serious concerns being expressed all over the world with respect to contamination and adulteration of traditional medicines. Contaminants may include various pesticides, herbicides, insecticides, heavy metals (arsenic, cadmium, lead, and mercury), microbial species, radiation, etc., and adulterants may include other (perhaps cheaper) plant materials with similar biological effects or synthetic drugs [5]. Microbial contamination can be controlled. It is a fact that natural materials harbor a large number of spores and other microorganisms. However, on the contrary, the maximum number of microorganisms allowed is regulated in most pharmacopeia [4].

Quality control is also very important to control side effects (plant extract toxicity). Botanical secondary compounds are not benign molecules. Ecologically speaking, these have evolved as chemical defenses that can repel, stun, poison, or kill other species. It would be easy on anybody's behalf to think that every plant extract is necessarily safe for human consumption. Nonetheless, it would be difficult for an inexperienced consumer to distinguish between effective medicines from deadly poisons. Over and above the dosage factor, it is adulteration either accidentally or intentionally.

It is not a simple exercise of applying modern technologies to quality control of the products that have been in constant use for centuries. New technologies for botanical authentication, phytochemical analysis, and biological determination are employed for the development of new strategies to improve the quality control of medicinal plants [4]. Microscopy is less important here as opposed to phytochemical methods. In the case of the herbal extracts, Thin-Layer Chromatography is only feasible for some components, as the flavonoids, while the presence of vital minor compounds may be masked and may not be very visible. It is in this particular instance that the complementarity of analytical methods as HPLC are of paramount importance for analyzing both the lead and the minor compounds, as polyphenols and vitamins.

The use of chromatographic fingerprinting demonstrates to be an effective and efficient tool for the bud-extract quality control of the available herbal products. The complex relationship between the chromatographic fingerprints and efficacy of the herbal medicines could also be one of the most important aspects for the herbal medicine quality control. Chemical fingerprints might be linked to biological assays to provide assurance of efficacy and consistency. In general, the research work on this aspect is far from sufficient to meet the needed criteria: the studies on the analytical fingerprint of herbal preparations in relation to their efficacy are an important task for quality control of these products. At the same time, it is urgent to evaluate

the possible contaminations in phytotherapeutic medicines (e.g., pesticides, microbial agents, heavy metals, and toxins).

4.4. Phytochemical fingerprint

Few data are available on natural product quality (intended as safety and efficacy) because adequate and validated methods and health care policies for herbal medicine evaluation are still lacking. Currently, only one or two biomarkers were considered for the evaluation of herbal product quality and authenticity, and for the assessment of the quantitative chemical composition of a herbal preparations and in assessing the quantitative herbal composition of a herbal product [81, 44]. This kind of determination, however, does not give a complete picture of a herbal product because multiple constituents are usually responsible for its therapeutic effects: it is necessary a chemical fingerprint be obtained by several analytical techniques.

By definition, a chromatographic fingerprint of a herbal medicine is a chromatographic pattern of the extract of the most common pharmacologically active compounds. With the help of obtained chromatographic fingerprints, the authentication and identification of herbal medicines can be accurately conducted even if the amount and/or concentration of the chemically characteristic constituents are not exactly the same for different samples of this preparation: the chromatographic fingerprints could demonstrate both the “sameness” and “differences” between various samples successfully.

A herbal preparation may contain several natural compounds and, for this reason, a combination of different herbs could create interactions with many of these constituents during the extraction phases: therefore, a chromatographic fingerprint may present a complete representation of all the chemical molecules of a natural medicine.

The chromatographic technique allows obtaining a relatively complete profile of bud-preparations, the chromatographic fingerprint, representing the so-called phytocomplex. Obtaining a good chromatographic fingerprint representing the herbal preparation phytocomplex depended on several analytical factors, such as the extracting methods, the measurement instruments and the measurement conditions, according to other studies [66]. In particular, it is necessary to have a good extracting method, in order to obtain an informative fingerprint of herbal products with almost all the bioactive compounds to represent the integrity of the bud-preparations. Moreover, a chromatogram with good separation and a representative concentration profile of the bioactive components detected by a useful detector, as diode array detector, is also required.

A botanical fingerprint with all of its peaks just completely separated should be featured by maximal information content, and further separation cannot provide any more information and becomes unnecessary. It is a multivariate system, since in general it embraces most of the phytochemical constituents of herbal products [62]. Once different analytical fingerprints are obtained from the same herbal product or from different preparations, first of all it is important to evaluate any similarities and/or differences between them, also considering possible variation sources and their effects (e.g., retention time shift) from one chromatographic pattern to another. Moreover, the concentration profiles changes greatly for the herbal medicines

depending on the different producing places and/or harvest seasons and wrong results would have been obtained by simply seeking the optimal correlation coefficient between the chromatograms.

The construction of chromatographic fingerprints aims at evaluating the bud-product quality. Even if one or two markers or bioactive compounds are currently employed for evaluating the quality and authenticity of a herbal medicine, this determination does not give a complete picture of a herbal product. The fundamental reason of herbal medicine quality control is based on the concept of total herbal phytocomplex in order to identify the real herbal medicine and the false one [41]. If some fingerprints from different extraction methods and/or from different herbal medicines are considered, it is possible to see the same phytochemical constituents or to understand their bioactivities and possible side effects: it is very important for the quality assessment of different samples to determine the presence or absence of interested components among the different chromatographic fingerprints.

4.5. Case study: Phytochemicals in *Ribes nigrum* bud-preparations

Ribes nigrum L. (Family: *Grossulariaceae*) is commonly used as herbal medicine and it is popularized for its alleged tonic effect and possible curative and restorative properties; for this reason, blackcurrant bud-preparations, derived from embryonic fresh plant tissues, are important therapeutic remedies, prescribed in hepatic, respiratory, circulatory, and inflammatory disorders, but data on their chemical composition are lacking as, to date, phytochemical studies have principally been performed on barks, roots and root exudates, leaves, fruit, and seeds. The most important industrial product of *Ribes nigrum* is fruit; however, due to their particular chemical composition and excellent flavor, leaves and buds are also used in some applications as a raw material for the herbal and cosmetic industries: many people use the buds as medicinal preparation for their anti-inflammatory activity and antidermal diseases (eczema and psoriasis) [86].

By nature herbal preparations are complex matrices, comprising a multitude of compounds that are prone to variation due to environmental factors and manufacturing conditions. The current practice of identifying herbal extracts is by measuring the concentration of the main botanicals; their concentrations are used to characterize the herbal preparations and fingerprinting is recommended by the main pharmacopeias as a potential and reliable strategy for the quality control of complex mixtures.

The aim of this research was to perform an analytical study of *Ribes nigrum* bud-preparations, in order to identify and quantify the main bioactive compounds, obtaining a specific chemical fingerprint to evaluate the single class contribution to herbal preparation phytocomplex. The same analyses were performed using a High-Performance Liquid Chromatograph-Diode Array Detector (HPLC-DAD). In March 2013, samples of *Ribes nigrum* L. buds were picked up in a germplasm repository in San Secondo di Pinerolo, Turin Province (Italy): two different varieties (Rozenthal and Tenah) were sampled in different phenological stages (bud sleeping, bud break, first leaves), in order to test the genotype/sampling time effect on the chemical composition of the final product. Buds were used fresh to produce herbal preparations.

The protocol of bud-preparations is detailed in the monograph “Homeopathic preparations,” quoted in the *French Pharmacopoeia*, 8th edition, 1965 [29]. Bioactive compounds were extracted through a cold maceration process for 21 days, in a solution of ethanol (95%) and glycerol, followed by a first filtration, a manual pressing and, after two days of decanting, a second filtration. Macerated samples were then stored at N.A., at 4°C and 95% R.H. Different chromatographic methods were used to analyze the macerated samples, two for polyphenols and one for terpenic compounds, organic acids, and vitamins, respectively. Mineral elements were also considered.

The content of total bioactive compounds in the evaluated bud-preparations is reported in Figure 4. Statistically significant differences were observed among the analyzed samples depending on genotypes and bud phenological stages.

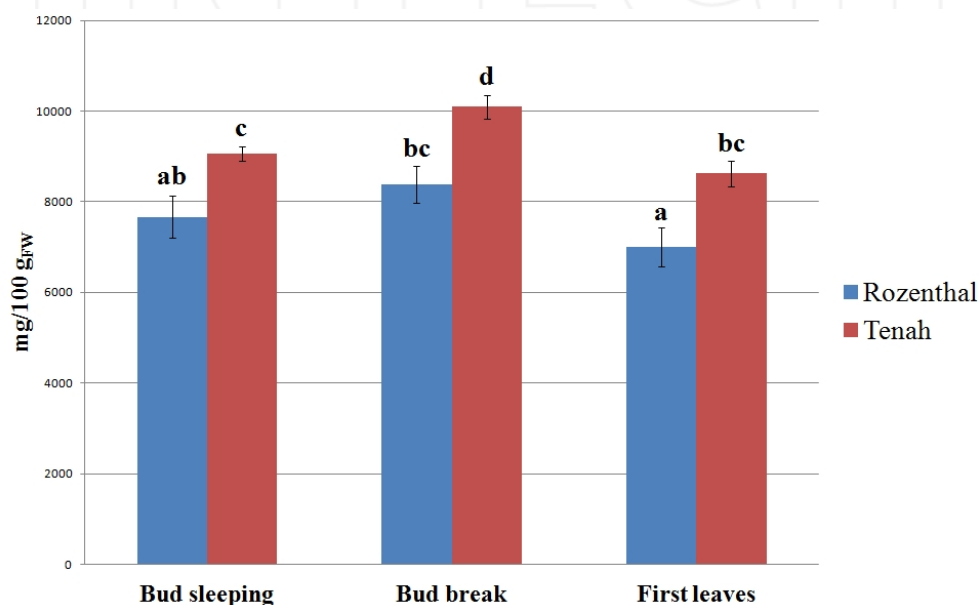


Figure 4. Total bioactive compound content in *Ribes nigrum* bud-preparations.

Ribes nigrum was identified as a rich source of anti-inflammatory and antioxidant compounds: the observed analytical fingerprint demonstrated that these bud-preparations represent a rich source of organic acids, terpenic and polyphenolic compounds, especially catechins and phenolic acids (Table 3).

Chemical fingerprint showed the prevalence of organic acids and monoterpenes in chemical composition of all the analyzed samples (mean values were considered): the most important class was organic acids (75.57%), followed by monoterpenes (18.45%), polyphenolic compounds (4.94%), vitamins (0.95%), and mineral elements (0.09%) (Figure 5).

Bioactive Compounds		
mg/100 g _{FW}		
Benzoic Acids	<i>gallic acid</i>	46.018
	<i>ellagic acid</i>	34.270
Cinnamic acids	<i>caffeic acid</i>	1.757
	<i>chlorogenic acid</i>	20.354
	<i>coumaric acid</i>	7.645
	<i>ferulic acid</i>	87.175
Catechins	<i>catechin</i>	53.026
	<i>epicatechin</i>	63.501
Flavonols	<i>hyperoside</i>	n.d.
	<i>isoquercitrin</i>	1.801
	<i>quercetin</i>	68.921
	<i>quercitrin</i>	34.066
	<i>rutin</i>	n.d.
Mineral elements	<i>calcium</i>	0.701
	<i>iron</i>	0.165
	<i>magnesium</i>	0.697
	<i>phosphorus</i>	1.626
	<i>potassium</i>	4.694
Monoterpenes	<i>limonene</i>	121.583
	<i>phellandrene</i>	163.176
	<i>sabinene</i>	27.607
	<i>γ-terpinene</i>	57.633
	<i>terpinolene</i>	1194.037
Organic acids	<i>citric acid</i>	28.063
	<i>malic acid</i>	2189.727
	<i>oxalic acid</i>	57.434
	<i>quinic acid</i>	3259.735
	<i>succinic acid</i>	481.857
Vitamins	<i>tartaric acid</i>	391.420
	<i>B1</i>	0.015
	<i>B2</i>	0.002
	<i>C</i>	80.747

Table 3. Information relating to botanical fingerprint of *Ribes nigrum* bud-preparations.

Analytical fingerprinting could be an important tool to study the assessment of chemical composition and bioactivities of plant-derived products, helping in finding new sources of natural health-promoting compounds: this study allowed to develop an effective tool for quality control through the botanical fingerprinting of bud-preparations.

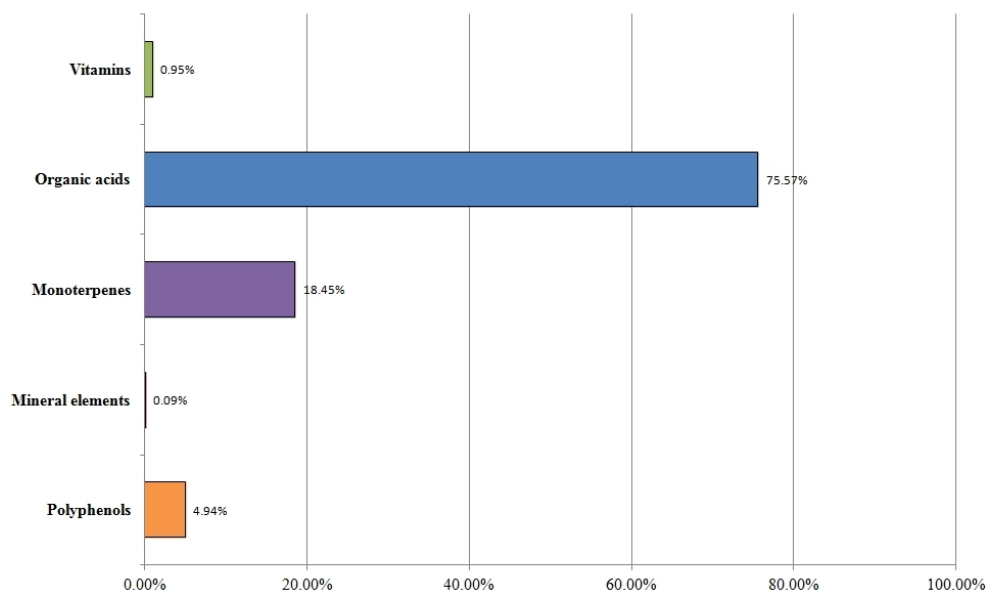


Figure 5. *Ribes nigrum* bud-preparation phytocomplex.

5. Conclusion

5.1. Herbal preparation supply chain

The creation of specific horticultural models for bud-production could be an interesting opportunity to obtain a high standardized raw material for herbal preparations. If natural products are to be produced for utilization in the pharmaceutical industry, there will be certain criteria that a system of production must meet. First, it clearly must be economical: if the drug costs hundreds to thousands of dollars per dose, there is no viable product [39].

The production system must be sustainable and reliable because the plant part has to be harvested directly from its source (thus endangering the survival of the species), and the natural compound extraction may not be economically viable [8]. A source of bioactive compounds must be available to meet different medical needs, but today's society requires that production of natural products to be environmentally safe and nonenvironmentally impacting. Alternative methods for herbal preparation production include cultivation of the target species, synthesis, and biotech production. These approaches closely follow the original phytochemical study in order to assess the quality and yield of the production and the purity of the final product, but to be economical, these methods must also include fast analytical techniques.

To meet these criteria and establish a viable production system, all the steps of production of a natural product must be systematically evaluated. First, a source of bioactive substances must be identified: cultivars of the same species must be considered in order to choose the highest and the most stable concentration of the natural compounds. Once this source has been identified, an uninterrupted and stable supply of plant material must be secured. This means that an agronomic system for biomass production must be developed in order to allow the full expression of the genetic capability of the cultivar.

Climate and soil should fit with the species requirements. The impact of fertilization and irrigation on the biomass production and its chemical constituents must be understood and, generally, the economic growth and cultivation of the raw material must be explored [53]. In the future, a plant production of secondary metabolites may be controlled with different growth regulators according to Good Agriculture Practices (G.A.P.) used in this agronomic production [90].

The successful production of biomass should include the development of appropriate harvest techniques. Topics to consider are the determination of the best bioactive compound concentration in the plant material during the growing season and the handling of freshly harvested biomass to retain bioactive content: the choice of the best phenological stage during bud-harvesting period is a fundamental step to obtain high values of bioactive compounds in bud-products. Moreover, mechanization of the harvest process must be addressed in order to maintain an economic system of production. Harvested biomass should require an economic processing facility in order to process biomass for the plant material isolation over an entire calendar year, not just immediately after harvesting. Therefore, technology should be developed to stabilize the biomass so that it retains bioactive content during storage before its ultimate processing: this usually involves developing an appropriate drying process. For this reason, analytical fingerprint could be also an important tool to evaluate the bioactive compound stability during product storage.

Finally, once the biomass processing is initiated, the extraction–purification system should be economical and efficient in its recovery of the bioactive compounds from the biomass. Moreover, it should be safe in its operation, and the generation of waste products should be minimized so that there is no deleterious environmental impact from the material processing.

5.2. Directions for further studies

Research into medicinal plants and the search for plant-derived preparations require a multidisciplinary approach with integrated projects, financial and technical support, and a very carefully planned strategy [12]. The research field of herbal medicine quality control is really an interdisciplinary research: it needs a crossover of chemistry, agricultural science, pharmacology, medicine, and even statistics to provide a platform for the quality control of herbal products and further discovery of the novel therapeutics composed of multiple chemical compounds [44]. The aims should consider demands in terms of public health, preservation of Biodiversity and the technical qualification of each laboratory or research group involved ; advances in technology and knowledge of natural products should be viewed not merely from the perspective of drug development, but also as a special tool for the understanding of

biological phenomenon in order to contribute to the well-being of humanity [4]. Chemical analysis of extracts from plant material could play a central role in the development and modernization of natural medicines, botanicals and herbal preparations.

In order to evaluate the entire substances pattern of complex bud-products, chromatography offers a powerful tool for separating the individual compounds and hereby creates a specific fingerprint profile. Later on, when the analyses are performed, data handling methods should be used to extract the useful information residing within the enormous amounts of generated data. The use of an experimental design for the selection of the most influential factors and their optimization is important.

Once the analytical method is established, the herbal fingerprints can be recorded. Although major improvements in the quality control of herbal medicines have been made, proper fingerprint analysis does not only include an adequate choice of the analytical technique [40]. For this reason, more attention has been paid to the optimization of the extraction and separation procedures using appropriate experimental designs, even if an adequate preprocessing of the fingerprints and a critical validation of the results obtained by the data handling techniques are too often neglected [41].

Compounds as the macromolecules (e.g., polysaccharides), a major part of the bioactive compounds in herbal species, are often not considered in herbal fingerprinting, and should be taken into account during future development of quality control criteria. For this, several innovative methods based on the interaction with biological systems and dialysis methods are at the point of breakthrough and could allow scientists to approach quality control issues from other angles, reducing the complexity of the matrix and focusing on the directly related biologically active compounds. Accordingly, the biological activity of herbal samples can be integrated in the fingerprint development, focusing on the relevant biological information.

Just as for pharmaceutical products, a well-validated method can help in monitoring the stability of the botanicals and herbal preparations over a period of time: the fingerprint could be also applied in this direction. It is likely that the combination of chemical standardization with biological assay could provide further knowledge about therapeutic effects of the medicinal plants [37].

Finally, the use of validated methods in the chemical standardization of botanicals and herbal preparations could enhance the quality of the products, assist in pharmacological studies, perform credible clinical trials and propel the move toward evidence-based medicine.

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